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Application of electron beam irradiation for inhibition of *Fusarium oxysporum* f. sp. *dianthi* activity

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Abstract. Electron beam irradiation was tested against *Fusarium oxysporum* f. sp. *dianthi* (*Fod*) a pathogen causing *Fusarium* wilt of carnation. Efficiency of the different radiation doses on *in vitro* survival and development of *Fod* culture on potato-dextrose agar (PDA) medium was tested. A dose of 6 kGy completely inhibited the pathogen growth. Application of radiation for microbiological decontamination of four substrates used for carnation production demonstrated that, depending on the type of substrate, doses of 10 or 25 kGy were effective in *Fod* elimination. All carnation plants cultivated on radiation decontaminated substrates were healthy.

Key words: electron beam irradiation • *Fusarium oxysporum* • substrates microbiological decontamination • carnation • healthiness

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Introduction

Fusarium wilt and *Fusarium* rot caused by formae specialis *Fusarium oxysporum* Schlecht are the most dangerous plant diseases. Among them *Fusarium* wilt of carnation (*Dianthus caryophyllus*) incited by *F. oxysporum* Schlecht f. sp. *dianthi* Sny et Hans (*Fod*) is the most destructive pathogen of that plant [7]. There are two main sources of the pathogen: carnation cuttings taken from mother plants looking healthy but already with vessels infected by *Fod* and substrates used in previous production infested by the pathogen [7]. During the last 20 years, peat or its mixture with composted pine bark were mainly used for carnation growth [9]. Introduction of new plant protection products, especially benzimidazoles decreased only partly plant losses [6, 7]. The use of steaming for elimination of *Fod* from infested substrates is expensive and acts destructively on peat structure. Application of dazomet for substrates disinfection needs at least 4 weeks in the case of peat and composted pine bark at about 18°C, while its efficacy is about 80% [3]. In such a situation searching for simply adapted and fast decontamination methods of substrates is necessary. The above-mentioned requirements can be fulfilled by applying ionizing radiation. This technology is commercially used for sterilization of single use medical devices as well as for microbiological decontamination of food products [1]. Our preliminary results proved that irradiation of substrate could be an alternative method for *Fod* control. The purpose of the

present study was to (1) estimate the efficacy of different radiation doses on *in vitro* survival and development of *Fod* culture on artificial medium and (2) applicability of radiation treatment for microbiological decontamination of four substrates used for carnation production.

Materials and methods

Culture of *F. oxysporum* f. sp. *dianthi*

For *in vitro* trials and infestation of four substrates, isolate Gz 274 obtained from infected carnation vessel was used. Stock culture was grown on a PDA medium in 90 mm Petri dishes at 24°C in the dark.

Substrates

Perlite, peat, peat with composted pine bark (1:1) and composted pine bark were used. Substrates were stored in original bags in a greenhouse at 18–22°C.

Infestation of substrates

The culture for infestation of four substrates were grown on oats [8] in 90 mm Petri dishes twice sterilized in an autoclave at 120°C at intervals of 60 min. Plates were inoculated with 5 mm diam mycelial disks of *Fod* taken from 7 day old culture growing on PDA. After 2 week incubation at 24°C in the dark the contents of Petri dishes were homogenized with 250 ml of water/10 plate contents. The slurry was mixed with substrates in a concrete-mixer in a ratio of 1 plate content per 1 l of substrate. Such infested substrates were stored in bags at 18–22°C for 2 weeks. Before irradiation or treatment with dazomet, substrates were mixed again for about 15 min.

Irradiation

For irradiation, a linear electron accelerator ELEKTRONIKA was used [5]. The energy of electrons was 9 MeV. The cultures of *Fod,* incubated for 7 d inside 90 mm Petri dishes on the PDA medium at 24°C, were subsequently irradiated with doses of 0 (control), 1.5, 3.0, 4.5 and 6.0 kGy, respectively. For irradiation of substrates, the density of each sample was calculated to establish packaging pattern. The packaging is the main problem of electron beam (EB) irradiation processing especially for large volumes of soil. The substrates were irradiated in plastic bags (20 l each) with doses of 10, 25 and 40 kGy. The dose was measured with a graphite calorimeter and from a PCV dosimetric foil using dose reader CD-07 [2].

Relationship between radiation dose vs. survival and growth of *Fod* on PDA

Three hours after radiation treatment 5 mm diam. mycelial disks were taken from irradiated cultures and transferred on the PDA medium. Such a procedure was conducted during 20 d at 4 day intervals to evaluate the influence of radiation dose on survival, and if so, growth of *Fod*. After 4 day incubation, the diameter of colonies was measured on all growing cultures. In addition, to estimate the influence of radiation on *Fod* growth, 5 mm diam. mycelial disks were transferred from treated and untreated growing colonies on PDA and such a transplanting procedure was repeated three times. After 4 day incubation at 24°C, diam. of colonies were measured.

For estimation, the influence of radiation doses on spore germination of *Fod*, microconidia taken from untreated 7 day old cultures were transferred into sterilized, distilled water and spread on the surface of PDA in 90 mm Petri dishes. The plates were then irradiated with 0 (control), 1.5, 3, 4.5 and 6 kGy. After 24 h incubation, the number of colonies from germinated spores were counted. Experimental design was completely randomized with 4 replications (4 Petri dishes) while trials were repeated twice.

Estimation of *Fod* colony forming units (cfu) in irradiated and untreated substrata

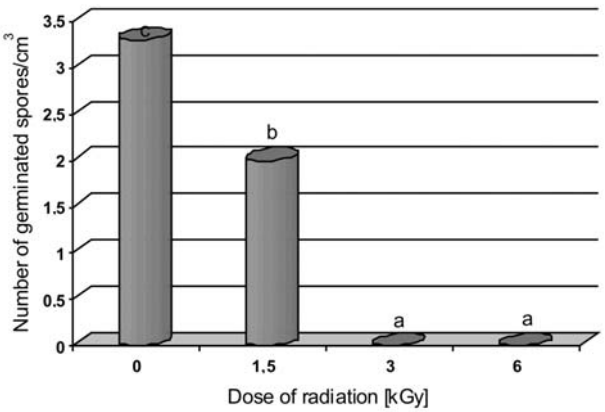
Komada [4] *Fusarium* selective medium and procedure described by Orlikowski [8] was used. Within 4 day incubation of medium seeded with substrates suspensions, the number of colonies growing were counted. Experimental design was completely randomized with four replication (4 plates) and trial was repeated twice.

Influence of substrates irradiation on health status of carnation

Rooted cuttings of cv. Master were planted into 1 dm^3 pots filled with irradiated substrates as well as to untreated (control, non-infested and infested with *Fod*) and disinfected with Nemazin 97 FG (97% of dazomet) at a concentration of 300 g/m^3. Pots were placed on a greenhouse bench and during 12 week growth at a temperature fluctuating between 18 and 27°C, the number of plants showing *Fusarium* wilt symptoms were counted. Experimental design was completely randomized with four replications and five plants in each experimental unit.

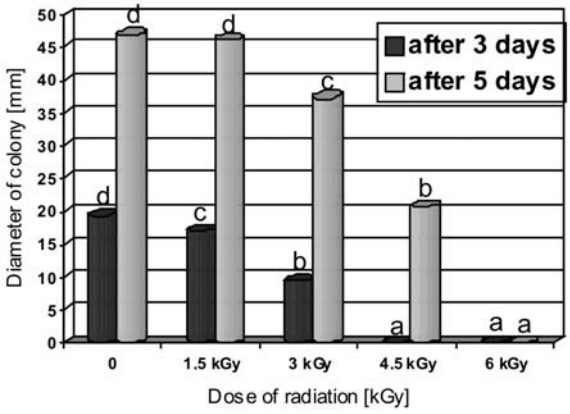
Results

Radiation treatment of Petri dishes with *Fod* spores seeded on PDA medium with 1.5 kGy resulted in a significant, about 40% decrease of survived conidia number. The increase of radiation dose to 3 kGy or higher (6 kGy) completely killed *Fod* spores (Fig. 1). Application of ionizing radiation to 7 day old cultures of *Fod*, growing on PDA in 90 mm Petri dishes, caused killing of the pathogen by a dose of 6 kGy. Transplanting of 5 mm diam. mycelium disks from the colonies irradiated with doses from 0 to 4.5 kGy resulted in a growth of *Fod*. There were no significant differences in the growth of untreated cultures and those irradiated with doses of 1.5 and 3 kGy. The colonies obtained from pieces of *Fod* irradiated with 4.5 kGy grown about twice slower than control pathogen (Fig. 2).



Note: Means signed by the same letter do not differ significantly (5%) acc from Duncan's multiple range test.

Fig. 1. Influence of irradiation on conidia survival of *Fusarium oxysporuum* f. sp. *dianthi*.



Note: See Fig. 1.

Fig. 2. Influence of *Fusarium oxysporum* f. sp. *dianthi* irradiation on their colonies growth.

Irradiation of four substrates infested with *Fod* resulted in a drastic decrease of cfu numbers (Table 1). The highest population density of *Fod* was noticed in untreated sample. In substrates irradiated with 10 kGy, the colonies of *Fod* were observed only on a selective medium amended with suspension of peat and peat with composted pine bark added. The number of colonies decreased, however, from about 27000 and 29000 to 300 cfu/g of air-dry substrate, respectively. The pathogen was not found in the infested substrates irradiated with the doses 25 and 40 kGy. The treatment of four substrates with dazomet resulted in a 10 fold reduction of cfu numbers (Table 1).

Growing of carnations in the infested substrates without treatment (control) resulted in a fast development of *Fusarium* wilt symptoms. First disease plants were noticed 7 weeks after planting on carnations in peat with composted pine bark. Within next week, *Fusarium* wilt was observed on plants growing in four tested substrates. After 10 weeks the disease occurred on at least 3/4 of carnations growing in perlite, peat and peat with composted pine bark and on about 2/5 plants in composted pine bark. Two weeks later most of control plants died or shown wilting symptoms (Table 2). Application of ionizing radiation (10 kGy) resulted in only a sporadic occurrence of *Fusarium* wilt on plants growing in radiation treated peat with composted pine bark. All carnations plants growing in substrates treated with two higher doses of radiation were healthy. The treatment of substrates with ionizing radiation eliminates pathogens and even at a dose of 40 kGy, which was estimated as maximum acceptable dose, did not affect negatively the plant growth. Application of dazomet for substrates treatment did not protected completely the plants against *Fusarium* wilt. After 3 month growth, the disease symptoms were observed on about 1/5 of carnation plants (Table 2).

Conclusion

Numerous methods can be used for soil sterilization in order to eliminate plant pathogens but usually they need application of chemical compound, long treatment time or extreme physical conditions (temperature, pressure).

Table 1. Number of colony forming units of *Fusarium oxysporum* f. sp. *dianthi*/g of air-dry substrate in relation to substrate type and radiation dose

Substrate type	Radiation doses (kGy)				Nemazin 97 FG 300 g/m^3
	0	10	25	40	
Perlite	27 700 b	0 a	0 a	0 a	1630 ab
Peat	27 100 b	300 b	0 a	0 a	2380 b
Peat + composted pine bark (1:1)	29 400 b	300 b	0 a	0 a	1230 a
Composted pine bark	17 900 a	0 a	0 a	0 a	1680 ab

Note: Means in columns, followed by the same letter, do not differ with 5% significance acc from Duncan's multiple range test.

Table 2. Development of *Fusarium* wilt of carnation cv. Master in relation to substrate type, radiation dose and growing period; number of diseased plants (n = 5)

Substrate	Radiation dose (kGy) and time (weeks) after planting								Nemazin 300 g/m^3	
	0		10		20		40			
	10	12	10	12	10	12	10	12	10	12
Perlite	3.0 b	4.8 b	0 a	0 a	0 a	0 a	0 a	0 a	0.5 a	1.3 a
Peat	3.8 b	5.0 b	0 a	0 a	0 a	0 a	0 a	0 a	0.5 a	0.8 a
Peat + composted pine bark (1:1)	3.0 b	4.8 b	0.5 a	0.5 a	0 a	0 a	0 a	0 a	0.8 a	1.0 a
Composted pine bark	1.8 a	3.8	0 a	0 a	0 a	0 a	0 a	0 a	0 a	1.3 a

Note: Means in columns, followed by the same letter, do not differ with 5% significance acc from Duncan's multiple range test.

In the present study radiation technology was used to eliminate *F. oxysporum* f. sp. *dianthi* from infested substrates used in carnation cultivation. Presented results show conclusively that irradiation of *Fod* culture on Petri dishes inhibits spores germination and growth of cultures. In the second experiment the effectiveness of radiation sterilization was proven in infested substrates used in plant growth. It is concluded that EB irradiation effectively eliminates *Fod* and protects carnations against the pathogen. Application of radiation sterilization technology was found highly effective as compared to other techniques (chemical methods) like application of dazomet, for example.

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